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Evaluation of an In-Packing Line Far-UVC Light System for Reducing Natural Microflora and *Escherichia coli* on Apple Surfaces

ABSTRACT

Recalls and outbreaks associated with apples, particularly related to postharvest handling, have pushed the industry to continually investigate microbial control techniques. Far-UVC radiation (UVC with shorter wavelengths) has potential for microbial inactivation with low risks to human health. This study was conducted to evaluate the efficacy of a far-UVC system (krypton chloride excimer lamp with peak emission of 222 nm) integrated into a packing line for reducing background microflora levels and artificially inoculated *Escherichia coli* on apple surfaces. Four far-UVC lights were previously installed in a two-by-two pattern on an apple packing line following spray bar sanitation. Two trials were conducted with 15 apples per treatment, assessing microbial levels before and after far-UVC treatment for 5 or 10 s at 0.3 m from apples on a roller bed. The light intensity above the roller beds was 237 μW/cm^2. Far-UVC treatment resulted in significant decreases in aerobic plate counts, total coliforms, yeasts, molds, and *E. coli* of 0.6, 0.6, 0.5, 0.9, and 0.7 log CFU per apple, respectively, indicating the potential for this treatment to enhance apple quality and serve as part of a hurdle approach for safety. Optimization and further research are warranted to fully harness the benefits of far-UVC technology for the apple industry.

INTRODUCTION

Historically, apples have been considered a low-risk commodity for foodborne pathogen contamination. However, in the past decade, they have been associated with numerous recalls *(31–35)* and outbreaks *(6)*, indicating their susceptibility to contamination. For example, in 2017 whole Gala, Fuji, Honeycrisp, and Golden Delicious apples that were bagged and individually sold were recalled when routine sampling revealed that finished products tested positive for *Listeria monocytogenes (33)*. In addition to dealing with microorganisms that pose public health risks, the apple industry must also address spoilage microorganisms in the postharvest environment. Decay caused by these microorganisms contributes significantly to the postharvest losses experienced by the apple packing industry in the Pacific Northwest *(20, 21)*. Therefore, identifying strategies

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and technologies to control microorganisms that impact both apple quality and the safety of the product during packing is crucial.

Chemical sanitizers have been widely adopted as a nonthermal technique used in the apple industry to help control cross-contamination of microorganisms in dump tanks and flume systems on apple packing lines. Chlorine is the most common sanitizer; it is cost-effective, readily available, and has minimal impact on the sensory and nutritional quality of produce *(8)*. Although antimicrobial agents such as chlorine are intended to reduce microbial cross-contamination in postharvest wash water, they do not act as a microbiological kill step for produce. Cross-contamination of apples due to equipment hygiene or sanitation practices would not be affected by antimicrobial agents in the wash water. Therefore, the apple industry is continuously seeking complementary strategies throughout the packing process as part of a hurdle approach for reducing microorganisms that impact apple safety and quality.

UVC radiation, with wavelengths of approximately 200 to 280 nm, has a strong germicidal effect against various microorganisms. Radiation, including UVC, is approved by the U.S. Food and Drug Administration for use in surface treatment of food under 21CFR179.39 *(30)*. UVC technology is easy to operate and cost-effective without leaving residues or generating by-products. However, the primary drawbacks of UVC radiation are its limited penetration ability and shadowing; areas obstructed from direct exposure do not receive adequate disinfection. This limitation makes UVC radiation best suitable for treating water, air, and flat surfaces where exposure is direct and unobstructed or for use in combination with other treatments as part of a hurdle approach. In the produce industry, UVC irradiation has been explored for decontamination of fresh-cut products, but its application to whole fresh produce is less common. Interest in the fresh produce industry is increasing in use of UVC radiation on packing lines to decrease background microflora levels on whole fruit and vegetable surfaces, thus reducing risks and improving the quality of produce. Traditionally, UVC systems use low-pressure mercury lamps with a peak emission of 254 nm. UVC radiation at 254 nm wavelength has been extensively documented as an effective technique for inactivating foodborne pathogens on produce and food-contact surfaces *(1, 7, 10, 12, 25, 29)*. However, exposure to UVC radiation at 254 nm poses significant safety concerns due to possible severe skin and eye damage *(11, 23)*, necessitating stringent worker safety measures.

Short-wavelength UVC (far-UVC) is a more recent focus based on its use with excimer lamps typically in the wavelength range of 200 to 230 nm. Far-UVC emitted from krypton chloride excimer lamps, with a peak emission of 222 nm, is of particular interest because of possibly reduced risks to human health compared with traditional UVC radiation at 254 nm *(13, 16, 37)*. Although the 222-nm wavelength of far-UVC is absorbed by the DNA and RNA of microorganisms, disrupting the replication process, it is unable to penetrate the outer cell layer of human skin or the outer tear layer of the eye *(5, 14, 27)*. Because of its potential use in occupied spaces, far-UVC radiation has been investigated as an air disinfection technology to reduce the spread of airborne pathogens. However, information on the efficacy of far-UVC treatment for food decontamination is limited, and to the authors' knowledge, no assessments of far-UVC radiation in a fresh produce processing line have been reported.

Responding to industry needs, the present study was conducted to evaluate the efficacy of a far-UVC system already integrated into an apple packing line. The system's capacity to decrease naturally occurring background microflora levels and artificially inoculated *Escherichia coli* on the surfaces of apples was assessed, providing an initial evaluation of the practical application of far-UVC technology in a produce packinghouse.

MATERIALS AND METHODS

Experimental design

Research trials were conducted at a packinghouse in central Washington state that previously installed four far-UVC lights (model 23F419, Sterilray, Somersworth, NH) on the packing line. The far-UVC lights were 0.635 m long (25 in.) and 38.1 mm (1.5 in.) in diameter with a maximum emission of 222 nm, 120 V, and 2.2 amps. These four far-UVC lights were arranged in a two-by-two pattern on the packing line, following the sanitizer spray bars and subsequent drying *(Figs. 1 and 2)*. The far-UVC lights were in a case cover positioned 0.3 m (12 in.) directly above the roller beds and had a UVC intensity of approximately 237 $\mu W/cm^2$ at the roller bed surface, as measured by a UVC meter (model 850010, Sper Scientific, Scottsdale, AZ). Fifty measurements were conducted with a stopwatch to determine how much time it took for apples to pass under the far-UVC light, with each light providing approximately 5 s of contact. Between the first and second far-UVC light was a gap of ca. 1 m.

Two independent trials were conducted on separate packing days with 15 unwaxed apples ('Honeycrisp' cultivar) collected per treatment group ($n = 30$) during each sampling event. The apples had been stored in controlled atmosphere conditions for approximately 6 months and were collected from the processing line 3 h into a typical packing day. For microbial analysis of uninoculated apples (i.e., aerobic plate counts, total coliforms, yeasts, and molds), apples were collected from the packing line (i) immediately after fan drying but before far-UVC treatment (control group), (ii) after treatment with one far-UVC light (5 s), and (iii) after treatment with two far-UVC lights (10 s). Nitrile gloves were worn for sample collection and changed between each apple sample.

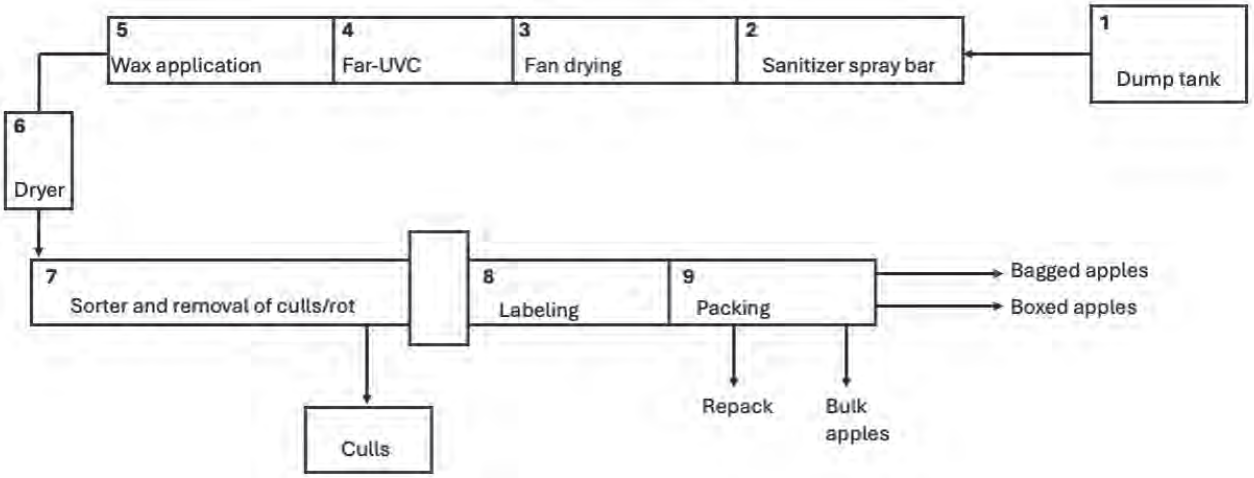

FIGURE 1. Flowchart of apples through the packing line.

FIGURE 2. (A) The two-by-two pattern of the far-UVC lights on the packaging line. (B) The 0.635 m long (25 in.) far-UVC lights were positioned 0.3 m (12 in.) above the roller bed.

For apples inoculated with *E. coli*, unwaxed Honeycrisp apples were collected from cold storage the day before inoculation and far-UVC treatment. After inoculation (described below), each apple was individually placed on the roller bed directly before the first far-UVC light, 3 h into a typical packing day. The apples were then exposed to either one far-UVC light (5 seconds) or two far-UVC lights (10 seconds), as described above. Since inoculated apples were placed on the packing line during a packing day, inoculated apples were marked to ensure recovery.

Enumeration of indicator organisms on apples

All apple samples were collected aseptically into sterile stomacher bags (Seward, Bohemia, NY) and immediately placed in a cooler on ice for transport. Each stomacher bag received 20 ml of 0.1% peptone water, and each apple was rubbed for 30 s, shaken for 30 s, and rubbed again for 30 s. Serial dilutions were performed in 0.1% peptone water, and 1 ml was surface plated in duplicate onto yeast/mold and *E. coli*/coliform petrifilm (3M, Saint Paul, MN). For aerobic plate counts, 0.1 ml was surface plated in duplicate onto tryptic soy agar (TSA; Thermo Fisher Scientific, Waltham, MA). To increase the limit of detection to 1.3 log CFU per apple, 1 ml of the lowest dilution was plated onto four plates (0.25 ml per plate) of TSA. TSA plates and *E. coli*/coliform petrifilms were incubated at 37°C for 24 and 48 h, respectively. Yeast/mold petrifilms were incubated at 24°C for 72 h. After incubation, yeasts, molds, *E. coli*, and coliforms were differentiated on petrifilm based on their distinct colors and morphology, as per the manufacturer's instructions.

Yeast colonies were small and blue-green with defined edges and no central foci. Mold colonies were large with diffuse edges and central foci. Total coliform colonies were red and blue with entrapped gas, and *E. coli* colonies were blue with entrapped gas.

E. coli inoculation, processing, and enumeration

The cocktail of *E. coli* used in the present study included three strains: TVS 353 (isolated from preharvest water), TVS 354 (isolated from lettuce), and TVS 355 (isolated from sandy loam soil) *(20)*. To distinguish them from the background *E. coli* on apple surfaces, the *E. coli* cocktail strains were adapted to grow in the presence of 80 µg/mL rifampin (Thermo Fisher Scientific) following a stepwise procedure *(18)*. Each *E. coli* strain was streaked from frozen stock (−80°C) onto TSA supplemented with 80 µg/mL rifampin (TSA-R) and incubated at 37°C for 24 h. After incubation, a 10-µl loopful of each strain was added into 10 ml of tryptic soy broth (TSB; Thermo Fisher Scientific) supplemented with 80 µg/mL rifampin (TSB-R) and incubated at 37°C for 24 h. Subsequently, 10 µl of these cultures was transferred into 10 ml of fresh TSB-R and again incubated at 37°C for 24 h. Each *E. coli* strain was added in equal volumes to reach a final inoculum cocktail level of 6 log CFU/ml. Each strain and the cocktail were enumerated on TSA-R to verify levels. For inoculation, 100 µl (10 spots of 10 µl each) of the *E. coli* cocktail was spot inoculated on the equator of each whole apple to reach a starting population of 4.0 ± 0.3 log CFU per apple. Inoculated apples were transported at 4°C to the packinghouse and used for the far-UVC experiments within 6 h of inoculation.

Inoculated apples were positioned individually on the roller bed and subjected to either one (5 s) or two (10 s) of far-UVC radiation. After the appropriate treatment duration, apple samples were aseptically collected into sterile stomacher bags and promptly transported to the laboratory on ice. To each stomacher bag, 20 ml of 0.1% peptone water was added, and apples were rubbed for 30 s, shaken for 30 s, and rubbed again for 30 s. Serial dilutions were made in 0.1% peptone water and surface plated (0.1 ml) in duplicate onto TSA-R. To increase the limit of detection to 1.3 log CFU per apple, an additional 1 ml of the lowest dilution was plated onto four plates each (0.25 ml per plate) of TSA-R. All plates were incubated at 37°C for 24 h, and *E. coli* was enumerated.

Statistical analysis

All analyses and data visualizations were conducted in R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Means and standard deviations of the log CFU per apple for each microorganism (i.e., aerobic plate counts, total coliforms, yeasts, molds, and *E. coli*) and treatment (i.e., no treatment, 5 s of exposure, and 10 s of exposure) were determined ($n = 30$). Results were evaluated with Tukey's honest significance difference test, and differences were considered significant at $P \leq 0.05$).

RESULTS AND DISCUSSION

After drying but before far-UVC treatment, unwaxed Honeycrisp apples on the packing line had aerobic plate counts and counts of total coliforms, yeasts, and molds of 4.0 ± 0.4, 3.6 ± 0.4, 4.2 ± 0.3, and 4.4 ± 0.4 log CFU per apple, respectively *(Fig. 3 and Supplemental Table S1)*. Although the bacterial and fungal populations on apples vary based on the enumeration method and length of storage, the populations of background microflora observed in this study were similar to those previously reported on apples *(9, 22, 26)*. High aerobic plate counts and counts of total coliforms, yeasts, and molds on apples during packing indicate a significant microbial load that can lead to spoilage and negatively impact apple quality, underscoring the need for effective technologies to reduce these populations.

Exposure for 5 s to a single far-UVC light positioned 0.3 m away from the apple surfaces resulted in significant reductions across all microbial populations. The aerobic plate count decreased to 3.5 ± 0.3, total coliforms to 3.1 ± 0.4, yeasts to 3.8 ± 0.2, and molds to 3.6 ± 0.4 log CFU per apple. Although further reduction in background microflora populations occurred on apples that passed through a second far-UVC light (10 s total), this change in populations was not significant compared with apples exposed to only one far-UVC light *(Fig. 3 and Table S1)*. The lack of a significant reduction with longer exposure times could be attributed to several factors, such as a saturation effect, where the remaining microbes had increased resistance to far-UVC radiation, or uneven light distribution to apples on the brush bed, preventing the radiation from reaching all microbial cells after the initial exposure. As apples move across a brush bed during processing, light exposure is not consistent across the entire surface of an apple because of irregular apple orientations, congestion on the bed, and collisions between apples, which can prevent them from rotating at a uniform rate or fully rotating at all.

Data on UVC treatments for reduction of background microflora populations that could reduce quality and safety of produce are limited. However, in a recent laboratory study of the effectiveness of 254-nm UVC versus far-UVC treatments for reducing conidia of the fungal pathogens *Botrytis cinerea, Penicillium expansum,* and *Colletotrichum* on strawberries, far-UVC was significantly more effective for reducing all three populations than was 254-nm UVC *(13)*. No negative effects were observed from the use of far-UVC treatment (at a distance of 30 cm and irradiated for up to 60 s) on strawberry plant photosynthesis, pollen tube germination, and growth, both in vitro and in situ *(13)*. Because the decay of apples is responsible for the majority of postpacking losses *(3)*, reduction of decay organisms within the background microflora would be extremely beneficial for maintaining apple quality and minimizing these losses. Previous results and those of the present study indicate that incorporation of far-UVC technology could significantly reduce populations of microbes, including those contributing to decay.

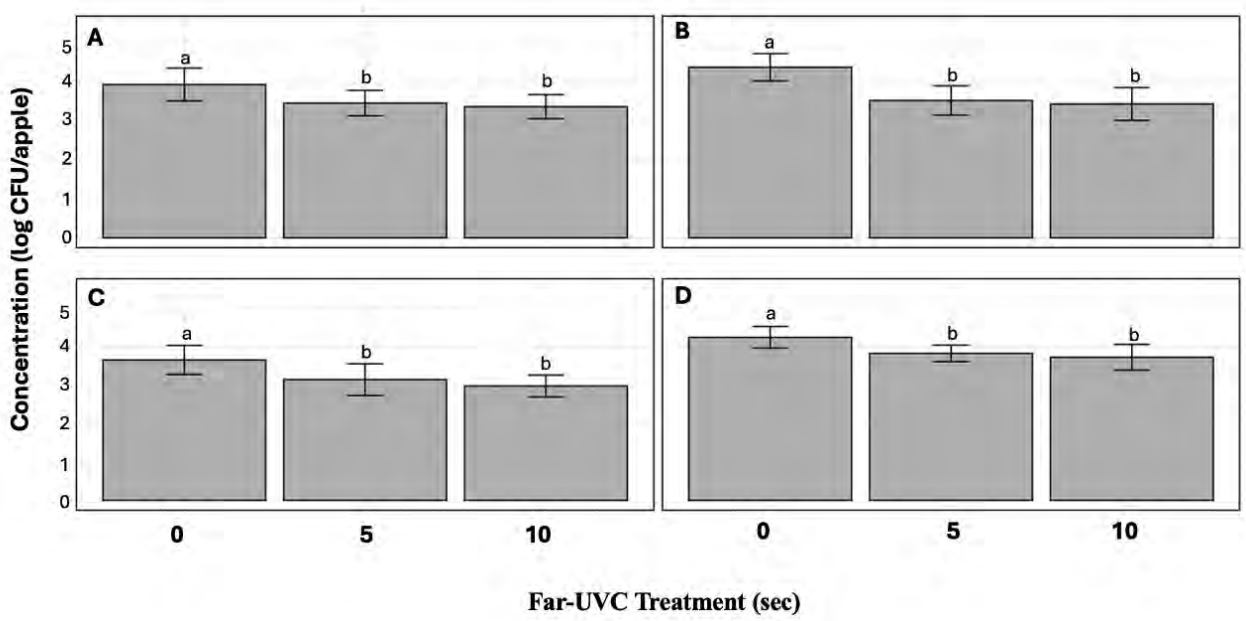

FIGURE 3. Population (log CFU per apple) of background microflora on Honeycrisp apples (n = 30) immediately before far-UVC treatment, after passing under one far-UVC light (0.3 m above, 5 s), and after passing under two far-UVC lights (0.3 m above, 10 s). (A) Aerobic plate count; (B) molds; (C) total coliforms; (D) yeasts. Population means with different letters were significantly different by Tukey's honest significance difference test.

TABLE S1. Populations of background microflora on Honeycrisp apple surfaces (n = 30) immediately before far-UVC treatment, after passing under one far-UVC light (0.3 m above, 5 s), and after passing under two far-UVC lights (0.3 m above, 10 s)[a]

Far-UVC treatment (s)	Aerobic plate count	Total coliforms	Yeasts	Molds
0	4.0 ± 0.4 A	3.6 ± 0.4 A	4.2 ± 0.3 A	4.4 ± 0.4 A
5	3.5 ± 0.3 B	3.1 ± 0.4 B	3.8 ± 0.2 B	3.6 ± 0.4 B
10	3.4 ± 0.3 B	3.0 ± 0.3 B	3.7 ± 0.3 B	3.5 ± 0.4 B

[a]Population means with different letters were significantly different by Tukey's honest significance difference test.

Because *E. coli* was detected on only 7.8% of apples (7 of 90), the impact of far-UVC on reducing *E. coli* populations could not be fully assessed; therefore, two inoculation studies were conducted *(Fig. 4 and Table S2)*. After exposure for 5 s to a single far-UVC light positioned 0.3 m away from inoculated apples on the packing line, the population of *E. coli* remained unchanged at 4.1 ± 0.3 log CFU per apple. However, passage under two far-UVC lights for a total of 10 s resulted in a significant reduction in the population of *E. coli* on apples to 3.3 ± 0.5 log CFU per apple. A significant reduction took longer to achieve on apples inoculated with the *E. coli* cocktail compared with apples with only surface background microflora. This difference may be due to survival of the dump tank and washing steps by the background microflora on the apples, making these microbes more susceptible to far-UVC treatment because of the injuries sustained during these processes. In contrast, the apples inoculated with *E. coli* were introduced to the line just before UVC exposure. The physiological state, sensitivity, and resistance of microorganisms to UVC irradiation at 254 nm varies depending on their cellular components, such as the structure, thickness, and composition of the cell wall *(17, 18)*. Consequently, variation among different microbial groups in their sensitivity to far-UVC irradiation at 222 nm is not unexpected.

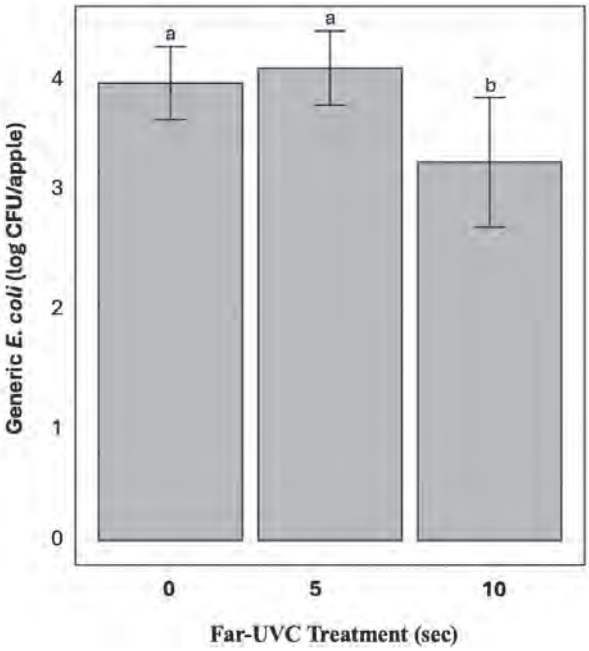

FIGURE 4. Population (log CFU per apple) of *E. coli* inoculated onto Honeycrisp apples (n = 30) immediately before far-UVC treatment, after passing under one far-UVC light (0.3 m above, 5 s), and after passing under two far-UVC lights (0.3 m above, 10 s). Population means with different letters were significantly different by Tukey's honest significance difference test.

TABLE S2. Populations of *E. coli* inoculated onto Honeycrisp apple surfaces (n = 30) immediately before far-UVC treatment, after passing under one far-UVC light (0.3 m above, 5 s), and after passing under two far-UVC lights (0.3 m above, 10 s)[a]

Far-UVC treatment (s)	*E. coli*
0	4.0 ± 0.3 A
5	4.1 ± 0.3 A
10	3.3 ± 0.5 B

[a]Population means with different letters were significantly different by Tukey's honest significance difference test.

In previous studies of the effects of far-UVC on *E. coli* and foodborne pathogens, significant reductions in populations have been found. In preliminary laboratory-scale research on tomatoes inoculated with *E. coli* (ATCC 25922) and exposed to 222-nm UV treatment at 3.40 ± 0.04 mJ/cm^2, a >4-log reduction was obtained *(2)*. Use of a 30-mJ/cm^2 treatment with 222-nm UVC radiation on water samples with turbidities of 0, 40, 80, and 120 NTU inoculated with *Salmonella* or *L. monocytogenes* at ca. 6 log CFU/ml resulted in surviving *Salmonella* populations of 0.00, 0.69, 2.91, and 3.24 log CFU/ml, respectively, and surviving *L. monocytogenes* populations of 0.00, 2.63, 5.45, and 5.65 log CFU/ml, respectively *(16)*. However, the radiation intensity used in these studies was considerably higher than that used in the present work. Higher radiation intensity is expected to achieve greater reductions in microbial populations under the same treatment conditions, such as those achieved on tomatoes and in water, replicating results in the postharvest environment (e.g., flumes). Increasing the radiation intensity and exposure uniformity for apples in the current system could lead to a higher radiation dose and thus more pronounced microbial reductions. Methods that could be used to enhance the performance of a far-UVC system include higher wattage lamps, improved radiation distribution across treated surfaces, and extended exposure times. Improved radiation distribution can be accomplished

by partially enclosing the areas containing the far-UVC lights with reflective materials, thus increasing coverage. The UVC exposure time on the dryer roller bed could be extended beyond the current 10 s by installing additional far-UVC lights to cover the entire brush bed, which is feasible within the commercial apple industry in the Pacific Northwest.

Although with far-UVC treatment resulted in significant reductions of 0.6, 0.6, 0.5, 0.9, and 0.7 log CFU per apple for aerobic plate counts and counts of total coliforms, yeasts, molds, and *E. coli*, respectively, these reductions did not reach the 1-log reduction required for effective lethal treatments. Consequently, under the given conditions the reductions were not practically significant. The effectiveness of far-UVC treatment for reducing microorganisms on the surface of produce can be influenced by several factors, including surface topography, exposure time, distance from the source, and produce movement and orientation on the conveyor, all of which affect the UVC dose the target microorganisms receive. The authors acknowledge that data on the far-UVC system's efficacy and optimal operating conditions in a produce operation were not available at the time of installation, underscoring the need to evaluate the effectiveness of this treatment in this context. The setup of the system evaluated does not allow for equal treatment of apples, given the placement of the lights *(Fig. 2)*. Future research focused on optimizing this technology and its implementation in produce operations should consider the effects of installing more far-UVC lights with higher intensity, extending treatment time, adjusting the distance and angle to the produce surface, and timing the treatment on the processing line (e.g., after the spray bar but before drying), which may all impact the overall performance of the far-UVC treatment. Other species of microorganisms of interest to the apple industry not examined in the present study (e.g., other *Listeria* species) may differ in their sensitivity to UVC treatments, and these difference should be further characterized to understand the full benefits of this technology. Because this research was used within a commercial setting, foodborne pathogens (e.g., *L. monocytogenes*) and indicator organisms (e.g., *Listeria innocua*) could not be used for the inoculation study. However, the three *E. coli* strains in the present were selected because of their origin in the produce production environment (e.g., water and soil) and are frequently used in field and greenhouse experiments where pathogenic strains cannot be used *(4, 15, 23, 36)*. Future laboratory research should investigate the reduction of *L. monocytogenes* on apples, with a particular focus on the stem and calyx areas.

Even under the nonoptimal conditions of this study, the integration of far-UVC radiation resulted in significant reductions in aerobic plate counts and counts of total coliforms, yeasts, molds, and *E. coli*. Therefore, leveraging far-UVC treatment with established techniques used by the apple industry, such as chemical sanitizers and controlled atmosphere storage, could synergistically enhance the overall quality and safety of apples. Further investigations are needed to (i) optimize far-UVC treatment in the apple processing line to maximize its dose and efficacy, (ii) evaluate the efficacy of far-UVC with other established postharvest techniques, (iii) observe the long-term effects of far-UVC exposure on human safety and fruit quality, and (iv) conduct a cost analysis covering installation, energy use, maintenance, and changes in decay losses. This information would help the industry make informed decisions about technology adoption, considering the decontamination efficacy, worker safety, and cost-effectiveness of UV technologies.

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